

# Coupled Enzymatic Production of Sulfite, Thiosulfate, and Hydrogen Sulfide from Sulfur: Purification and Properties of a Sulfur Oxygenase Reductase from the Facultatively Anaerobic Archaeobacterium *Desulfurolobus ambivalens*

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From aerobically grown cells of the extremely thermophilic, facultatively anaerobic chemolithoautotrophic archaeobacterium *Desulfurolobus ambivalens* (DSM 3772), a soluble sulfur oxygenase reductase (SOR) was purified which was not detectable in anaerobically grown cells. In the presence of oxygen but not under a hydrogen atmosphere, the enzyme simultaneously produced sulfite, thiosulfate, and hydrogen sulfide from sulfur. Nonenzymatic control experiments showed that thiosulfate was produced mainly in a chemical reaction between sulfite and sulfur. The maximum specific activity of the purified SOR in sulfite production was 10.6  $\mu\text{mol/mg}$  of protein at pH 7.4 and 85°C. The ratio of sulfite to hydrogen sulfide production was 5:4 in the presence of zinc ions. The temperature range of enzyme activity was 50 to 108°C, with a maximum at 85°C. The molecular mass of the native SOR was 550 kilodaltons, determined by gel filtration. It consisted of identical subunits with an apparent molecular mass of 40 kilodaltons in sodium dodecyl sulfate-gel electrophoresis. The particle diameter in electron micrographs was  $15 \pm 1.5$  nm. The enzyme activity was inhibited by the thiol-binding reagents *p*-chloromercuribenzoic acid, *N*-ethyl maleimide, and 2-iodoacetic acid and by flavin adenine dinucleotide,  $\text{Fe}^{3+}$ , and  $\text{Fe}^{2+}$ . It was not affected by  $\text{CN}^-$ ,  $\text{N}_3^-$ , or reduced glutathione.

Depending on the culture conditions, the facultatively anaerobic chemolithoautotrophic organisms *Desulfurolobus* and *Acidianus* spp. can grow either by oxidation of sulfur with oxygen, producing sulfate, or by anaerobic reduction of sulfur with hydrogen, producing  $\text{H}_2\text{S}$  (14, 28). Growth by oxidation of sulfur is a typical energy-yielding mechanism among the *Sulfolobus* group of thermophilic, sulfur-dependent archaeobacteria (15, 17). It is also common among the thiobacilli within the eubacterial kingdom (6). Chemolithoautotrophic growth by reduction of sulfur (S/H-autotrophy) is known only for the order *Thermoproteales* and the two above-mentioned genera among the *Sulfolobus* group of archaeobacteria (5, 14, 28) but has not yet been found within the eubacteria. The use of both pathways of energy conservation makes these organisms, *Acidianus* and *Desulfurolobus* spp., good candidates with which to study metabolic regulation mechanisms and the metabolic switch itself. As an initial step, I tried to isolate enzymes involved in sulfur oxidation or reduction.

The sulfur oxidation pathway consists of two steps, first the oxidation of sulfur to sulfite and then the oxidation of sulfite to sulfate (6, 25). Two different pathways of sulfur oxidation to sulfite have been found in mesophilic thiobacilli. From *Thiobacillus denitrificans*, a siroheme sulfite reductase oxidizing sulfur under anaerobic conditions was purified (11). Enzymes of the oxygenase type have been found in other thiobacilli (16, 19). Emmel et al. (4) recently presented evidence for the existence of a sulfur oxygenase in a freshly isolated strain of *Sulfolobus brierleyi* catalyzing the oxidation of elemental sulfur to sulfite with molecular oxygen. Only in one case has the disproportionation of sulfur to thiosulfate and hydrogen sulfide by an enzyme system from *Thiobacillus thiooxidans* been reported (21).

In this paper the isolation and characterization of a sulfur oxygenase reductase (SOR) from the facultative anaerobe

*Desulfurolobus ambivalens* is reported. The SOR was found only in aerobically growing, sulfur-oxidizing cells. It simultaneously produced sulfite, thiosulfate, and hydrogen sulfide from sulfur in the presence of oxygen.

## MATERIALS AND METHODS

**Organism and growth conditions.** *D. ambivalens* (DSM 3772), isolated and described by Zillig et al. (27, 28), was grown aerobically and anaerobically without organic substrates (28).

**Chemicals.** NADH, NADPH, and Tris were from Boehringer GmbH, Mannheim, Federal Republic of Germany. Sodium dodecyl sulfate (SDS) was from Roth, Karlsruhe, Federal Republic of Germany. Reduced glutathione, Tween 20, acrylamide, *N,N'*-methylenebisacrylamide (BIS), *N,N,N',N'*-tetramethylethylenediamine (TMED), and Coomassie brilliant blue were from Serva, Heidelberg, Federal Republic of Germany. Flavin adenine dinucleotide, *N,N*-dimethyl-*p*-phenylenediamine, *p*-chloromercuribenzoic acid, and *N*-ethylmaleimide were from Sigma, Munich, Federal Republic of Germany. Powdered sulfur (no. 7987; average diameter, 20  $\mu\text{m}$ ) was from E. Merck AG, Darmstadt, Federal Republic of Germany. All other chemicals (reagent grade) were also from Merck.

**Enzyme purification.** All steps were performed at 0 to 4°C. The bacteria were suspended in 9 ml of 20 mM Tris-acetate buffer (pH 8) per g of cells. They were disrupted by sonication for 5 min with a Branson sonifier (Branson Sonic Power Co., Danbury, Conn.) equipped with a microtip, and the pH was readjusted to 8.0 with a 2 M Tris solution. After DNase treatment for 30 min (DNase I; Boehringer), the crude extract was centrifuged for 60 min at  $100,000 \times g$  (SW 41 rotor; Beckman Instruments, Inc., Palo Alto, Calif.). The supernatant (S-100) was loaded on a linear sucrose gradient (28 to 40% [wt/wt]) in Tris-acetate buffer (800  $\mu\text{l}$  of S-

100/tube) and centrifuged for 48 h at  $290,000 \times g$  in an SW 41 rotor. The gradient was analyzed with a flow spectrometer (CE 212; Cecil Instruments, Cambridge, United Kingdom) at 280 nm. Fractions (1 ml) with the highest sulfur-oxidizing and reducing activities were collected and pooled.

Active fractions were further purified by horizontally run nondenaturing polyacrylamide gel electrophoresis (PAGE). Gels bound to gelBond PAG film (LKB, Bromma, Sweden) were cast in a chamber (250 by 200 by 3 mm). The spacer gel made of 3% (wt/vol) acrylamide, 20% (vol/vol) glycerol, 0.04% TMED, and 0.05% ammonium peroxodisulfate in 20 mM Tris-acetate buffer (pH 8.8) was cast first to a height of 5 cm, leaving a sample well (230 by 5 by 1.5 mm). The separation gel (4 to 15% gradient gel; acrylamide/BIS ratio, 30:0.8) was cast onto the spacer gel. The composition of the heavy solution was 4% (wt/vol) acrylamide, 20 mM Tris-acetate (pH 8.8), 12% (vol/vol) glycerol, 0.04% TMED, and 0.045% ammonium peroxodisulfate. The light solution was made of 15% acrylamide with the same buffer, TMED, and ammonium peroxodisulfate concentrations as above. Up to 10 mg of protein was separated on each gel. Gels were placed, as specified by the manufacturers, on the cooling block of a Multiphor II unit (LKB) and run at 4°C for 6 to 7 h, with 20 mM Tris-acetate (pH 8.8) for both electrodes. The power supply (ECPS 3000; Pharmacia, Uppsala, Sweden) was set to 3,000 V, 150 mA, and 50 W maximum. The actual voltage was 800 to 900 V initially and increased to 3,000 V at the end of each run. Alternatively, the gel was run for 14 h, with the power supply set to 800 V. The pH was 9.2 in the cathode buffer and 8.0 in the anode buffer at the end of the run.

After electrophoresis, a narrow strip was cut out of the gel and stained with Coomassie blue (8) for localizing protein bands. The active band was excised and electroeluted for 3 h in a dialysis tube in 20 mM Tris-acetate buffer (pH 8.8) with an electric field of 20 V/cm. The resulting enzyme solution was concentrated by lyophilization and finally dialyzed against 20 mM Tris-acetate buffer (pH 8).

**Enzyme assay for the SOR.** Sulfur (2%, wt/vol) was suspended in 70 mM Tris-acetate buffer (pH 7.4) with 0.005% Tween 20. The sulfur was dispersed by a 5-min sonication with a Branson sonifier at maximum power. The assay was performed in stoppered Hungate tubes shaken at 120 rpm. The pH curve was recorded by using 70 mM potassium phosphate buffer instead of Tris of the pH given (see Fig. 5). For every pH, nonenzymatic controls were run under identical conditions.

The reaction velocity was calculated from the slope of the maximal increase in concentration of sulfite plus thiosulfate or of hydrogen sulfide. One unit of activity was defined as micromoles of sulfite plus thiosulfate or of hydrogen sulfide formed per minute. Specific activities were expressed as units of sulfite plus thiosulfate formed per milligram of protein and units of hydrogen sulfide formed per milligram of protein. Protein was determined by the Coomassie blue method with bovine serum albumin (Bio-Rad Laboratories, Richmond, Calif.) for calibration (2).

The velocities of nonenzymatic reactions were determined by incubating sulfite and thiosulfate with a sulfur suspension (400 nmol/ml), omitting the enzyme, and determining the concentration of all three products after the time given. Controls under a hydrogen atmosphere were made anaerobic by evacuating the test tubes and flushing them with hydrogen gas six times each. Further control experiments were done by incubating the enzyme with sulfite and thiosulfate (400

nmol/ml), omitting the sulfur to investigate possible back reactions.

**Rhodanese assay.** Thiosulfate sulfurtransferase (rhodanese) activities of cell extracts and of the SOR were assayed at 80°C by the method of Wood and Kelly (26).

**Analytical procedures.** The sulfite concentration was determined by the method of Pachmayer (F. Pachmayer, doctoral dissertation, Universität Ludwig-Maximilians, Munich, Federal Republic of Germany, 1960) with slight modifications: 50  $\mu$ l of reagent (40 mg of fuchsin dissolved in 87.5 ml of double-distilled water and 12.5 ml of concentrated  $H_2SO_4$ ) and 195  $\mu$ l of double-distilled water were added to 250  $\mu$ l of sample. After 10 min at room temperature, 5  $\mu$ l of commercial Formalin was added. Readings were taken after at least a 90-min incubation at 570 nm against a reagent blank. The thiosulfate concentration was also determined by the method of Pachmayer (doctoral dissertation) by measuring the discoloration of methylene blue: 250  $\mu$ l of sample was added to 750  $\mu$ l of reagent (methylene blue dissolved in double-distilled water at 24 mg/liter and added to the same volume of concentrated HCl). Readings were taken after 24 h at 670 nm against a reagent blank. For the  $H_2S$  determination, the procedure of King and Morris (7) was followed, except that readings were taken after a 24-h incubation. Control experiments were done to study the effects of hydrogen sulfide and thiosulfate on the assays in mixed solutions. It was found, however, that the development of methylene blue in the assay for hydrogen sulfide was not affected by the presence of thiosulfate and that the bleaching of the dye in the thiosulfate assay was not affected by the presence of hydrogen sulfide, as a result of different reaction conditions. Tetrathionate was determined after cyanolysis (9), and sulfate was determined turbidimetrically after acidification (3).

**Molecular mass determination.** The molecular mass of the native enzyme was determined by gel filtration over a Bio-Gel A 1.5M column (2.4 by 60 cm; Bio-Rad). A molecular mass calibration kit (28 to 650 kilodaltons [kDa] Sigma) was used. The molecular mass of the monomeric subunit was determined by SDS-PAGE (10) in a 7.5 to 22.5% gradient gel with a 4.5% spacer gel. For calibration, lysozyme (14 kDa; Biomol Feinchemikalien GmbH, Hamburg, Federal Republic of Germany), carbonic anhydrase (28 kDa; Sigma), and bovine serum albumin (67 kDa; Bio-Rad) were used. Gels were stained with Coomassie brilliant blue (8).

**Inhibition studies.** The following chemicals were tested for inhibitory effects on the enzyme preparation:  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Zn^{2+}$ , KCN,  $NaN_3$ , flavin adenine dinucleotide, reduced glutathione, 2-iodoacetic acid, *N*-ethyl maleimide, and *p*-chloromercuribenzoic acid. All tests were done after an incubation time of at least 10 min at room temperature against a control tube containing the reaction mixture plus the substance omitting the enzyme, and against the uninhibited enzyme reaction.

## RESULTS

**Enzyme purification.** From cytoplasmic fractions of aerobically grown cells of *D. ambivalens*, a sulfur-oxidizing, sulfite- and thiosulfate-producing enzyme could be purified. Surprisingly, considerable amounts of hydrogen sulfide were also formed. Both activities copurified over the whole procedure and could not be separated. Particle-free extracts of anaerobically grown cells had no sulfur-oxidizing or reducing activity.

After cell debris and membrane fractions had been pelleted, most of the enzyme activity remained in the clear,

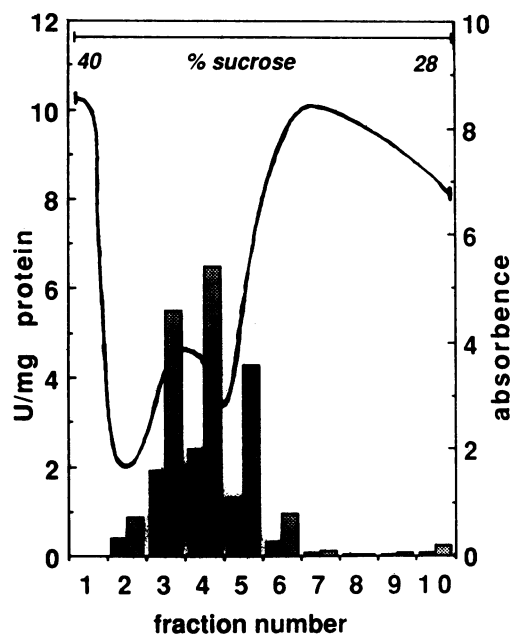


FIG. 1. Elution diagram of the sucrose density gradient centrifugation and specific activities of the resulting fractions. Left axis: enzyme activity; right axis:  $A_{280}$ . Symbols: ■, hydrogen sulfide production; ▨, sulfite plus thiosulfate production.

dark-yellow supernatant. In the sucrose density gradient the sulfur-oxidizing and reducing activities were highest in the minor peak (Fig. 1). Figure 2 shows a longitudinal section of a nondenaturing, preparative gel run in the next step of the purification procedure. Only the most prominent protein

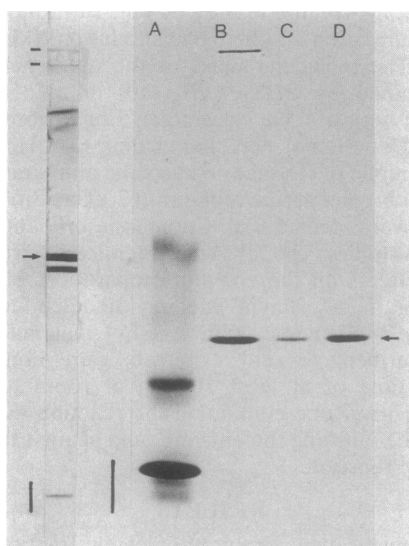


FIG. 2. Polyacrylamide gels of the SOR. Bars, 1 cm. (Left) Longitudinal section of a preparative nondenaturing gel loaded with fraction 4 from the density gradient centrifugation. The arrow indicates the position of the SOR; dashes at the top indicate the loading area of the gel. The lowest protein band was yellow; it was used as marker in electrophoresis. (Right) SDS-PAGE. Lanes: A, molecular weight markers (from top, bovine serum albumin [67,000], carbonic anhydrase [28,000], and lysozyme [14,000]; B, fraction 4 of the density gradient centrifugation; C, gel eluate; D, concentrated SOR.

TABLE 1. Purification yields of the SOR of *D. ambivalens*

Fraction	Amt of protein		Activity <sup>a</sup> (U/mg)	Yield	
	mg/ml	Total/ mg <sup>b</sup>		Total/ U <sup>b</sup>	%
Thiosulfate + sulfite formation					
Crude extract	87.7	649	1.89	1,310	100
S-100	60.0	432	2.08	897.3	68.5
Fraction 4 <sup>c</sup>	1.86	18.7	6.51	121.6	9.3
Gel eluate	0.19	9.6	10.6	101.2	7.7
Hydrogen sulfide formation					
Crude extract	87.7	649	0.50	327.1	100
S-100	60.0	432	0.74	319.7	97.7
Fraction 4 <sup>c</sup>	1.86	18.7	2.34	43.7	13.3
Gel eluate	0.19	9.6	2.69	25.8	7.9

<sup>a</sup> At 85°C.

<sup>b</sup> Per gram (wet weight) of cells.

<sup>c</sup> From density gradient centrifugation.

band had sulfur-oxidizing and reducing activities. After band excision and electroelution, the resulting protein solution was electrophoretically homogeneous, giving only one sharp band in SDS-PAGE (Fig. 2). A summary of the purification procedure is given in Table 1.

**Enzyme structure and stability.** The molecular mass of the intact enzyme was estimated by gel filtration to be 550 kDa. In SDS-PAGE the SOR proved to consist of identical subunits with an apparent molecular mass of 40 kDa (Fig. 2). UV-visible spectra of the enzyme solution in the oxidized and in the dithionite-reduced state gave no absorbance beyond the peak at 280 nm due to aromatic amino acid side chains (data not shown). The method for hydrogen sulfide determination (see Materials and methods) (7) revealed no acid-labile sulfur.

In electron micrographs of enzyme preparations, round particles of  $15 \pm 1.5$  nm in diameter were observed (Fig. 3). Two different types of particles of identical diameter became visible; one type had a dark dot in the middle, whereas the other did not have this structure (Fig. 3).

The SOR was partially inactivated by freezing at  $-80^{\circ}\text{C}$ . A preparation lost more than 80% of activity after three freezing and thawing steps. The purification was therefore performed without intermediate freezing.

**Enzyme products and activity.** Detectable products of enzyme activity were sulfite, thiosulfate, and hydrogen sulfide. Sulfate and tetrathionate were not found. The activity under a hydrogen atmosphere was very low. Figures 4a and b show an enzyme activity experiment and a control experiment under a hydrogen atmosphere. When SOR was incubated with sulfite or thiosulfate, no evidence for a possible back-reaction was found. The specific activities of different preparations varied considerably. In some cases, specific activities of more than 20 U/mg of protein (in density gradient fractions) were observed. The purified SOR did not have any rhodanese activity under the assay conditions. Rhodanese activity in cell extracts of aerobically grown cells was 9.1 mU/mg of protein; that in extracts of anaerobically grown cells was 3.3 mU/mg (1 U was defined as the number of micromoles of  $\text{SCN}^-$  formed per minute under the assay conditions).

Sulfite incubated with sulfur in a nonenzymatic control experiment at  $75^{\circ}\text{C}$  was converted almost quantitatively to thiosulfate within 3 min. No hydrogen sulfide was formed during this reaction (data not shown). Incubation of thiosul-

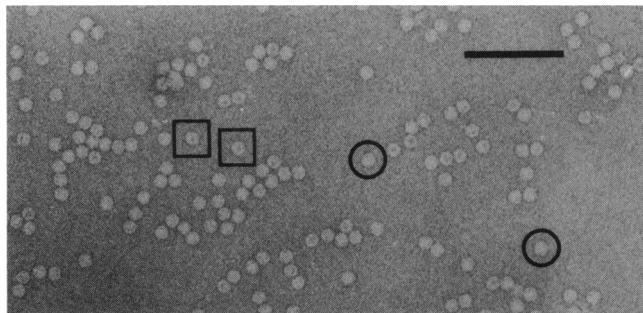


FIG. 3. Electron micrograph of the purified SOR, negatively stained with ammonium molybdate. Circles indicate intact enzyme molecules, squares indicate molecules with a dark dot in the middle. The latter particles might be enzyme molecules partially disintegrated during the purification process. This assumption would explain the low purification factors observed. Bar, 0.1  $\mu\text{m}$ .

fate with a sulfur suspension gave no decrease in thiosulfate concentration after 20 min, nor was any hydrogen sulfide produced. A small amount of sulfite initially present (impurities up to 0.5% in the thiosulfate [Merck]) also converted mostly to thiosulfate (data not shown).

**Stoichiometry.** The ratio between thiosulfate plus sulfite and hydrogen sulfide production catalyzed by crude extracts was 1.23:1 to 1.30:1 in several experiments when zinc acetate was present in the reaction mixture (Fig. 4c). Hydrogen sulfide production was linear from the beginning. Thiosulfate plus sulfite production showed a marked lag phase, but after becoming linear, it exceeded hydrogen sulfide production (Fig. 4). Omission of Tween 20 from the enzyme assay did not alter the sulfite/hydrogen sulfide ratio.

**Inhibition studies.** Inhibiting or stimulating effects of various substances are summarized in Table 2. Most interesting was the stabilizing effect of  $\text{Zn}^{2+}$  on hydrogen sulfide formation in crude extracts (disrupted cells and S-100), whereas

purified enzyme preparations (homogeneous preparations after electrophoresis and sucrose density gradient fractions) were inhibited.

**pH and temperature dependence.** The pH dependence of the enzyme activity and chemical controls at each pH is shown in Fig. 5. It was not possible to distinguish between enzymatic and nonenzymatic formation of thiosulfate at pH 9. The temperature dependence of the enzyme activity is shown in Fig. 6. At all temperatures, formation of sulfite, thiosulfate, and hydrogen sulfide was parallel (Fig. 6). At 112°C a considerable amount of chemically formed sulfite and thiosulfate was observed (20 nmol/ml per min under the assay conditions).

## DISCUSSION

A soluble SOR was purified from the extremely thermoacidophilic archaebacterium *D. ambivalens*. It proved to be solely responsible for the simultaneous reduction and oxidation of elemental sulfur under the assay conditions. By its structure (550 kDa, composed of identical subunits of 40 kDa), the similar purification procedure, and the sulfur-oxidizing activity, it resembled the sulfur oxygenase (560 kDa, identical subunits of 35 kDa) described by Emmel et al. (4). The most striking difference of the SOR was its sulfur-reducing activity. From results of nonenzymatic control experiments, it was concluded that  $\text{H}_2\text{S}$  production was enzyme dependent. Thiosulfate was formed mostly nonenzymatically, although no final decision could be made about whether it was also a primary reaction product of the SOR.

Views about the initial step of sulfur oxidation differed considerably from the results of various studies (6, 25). In phototrophic members of the family *Chromatiaceae*, siroheme sulfite reductases were found (12), similar to those of *Thiobacillus denitrificans* (11) and of various *Desulfovibrio* species (25), which were acting on sulfide, sulfur, and polysulfides formed nonenzymatically from sulfur and  $\text{H}_2\text{S}$  (25). Because of its structure and the lack of any absorbance

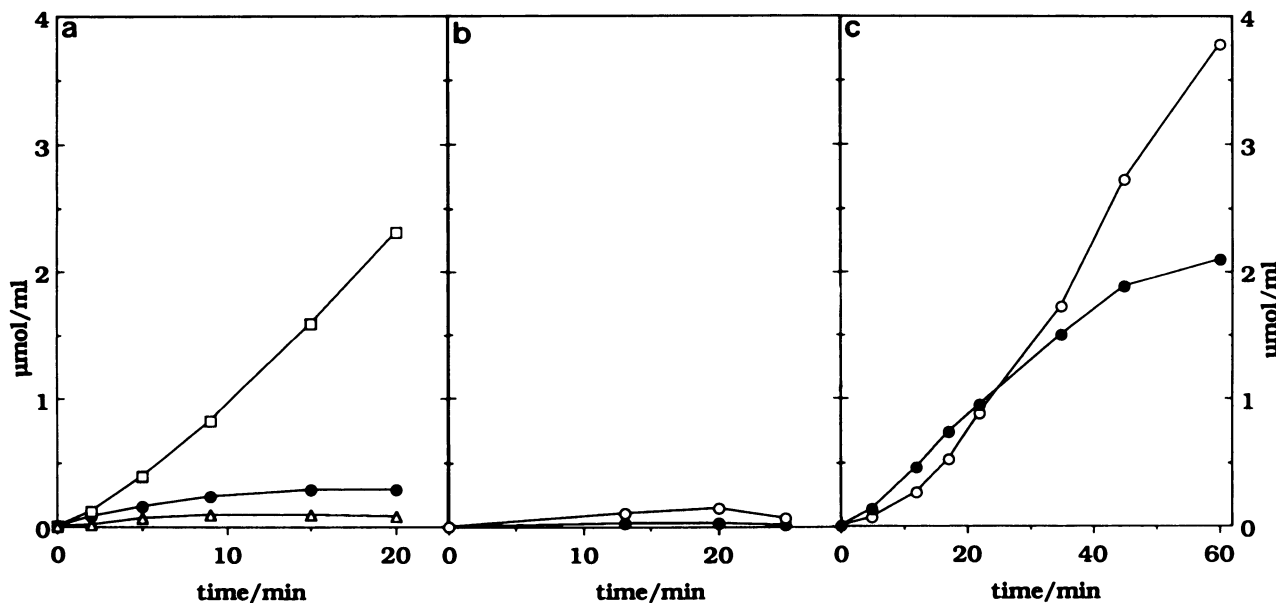


FIG. 4. Enzymatic activity of the SOR at 85°C as described in Materials and Methods. (a) Enzymatic production (11.2  $\mu\text{g}$  of protein) of sulfite, thiosulfate, and hydrogen sulfide from sulfur. Symbols:  $\square$ , sulfite;  $\bullet$ , hydrogen sulfide;  $\triangle$ , thiosulfate. (b) Control under hydrogen atmosphere. Symbols:  $\circ$ , sulfite plus thiosulfate;  $\bullet$ , hydrogen sulfide. (c) Activity of crude extract (124  $\mu\text{g}$  of protein in the presence of 5 mM zinc acetate). Symbols are the same as in panel b.

TABLE 2. Effect of inhibitors

Substance	Concn (mM)	% Activity	
		SO <sub>3</sub> <sup>2-</sup>	H <sub>2</sub> S
None		100	100
NaN <sub>3</sub>	5	94	110
KCN	1.0	101	117
	5.0	87	109
Fe <sup>2+</sup>	0.2	99	131
	1.0	56	115
Fe <sup>3+</sup>	1.0	86	160
	1.0	86	160
Zn <sup>2+</sup>	0.01	118	281
	0.1	74	144
	1.0	0	0
Zn <sup>2+</sup> <sup>a</sup>	0.2	116	145
	1.0	130	326
FAD <sup>b</sup>	0.1	104	98
	1.0	32	26
GSH	1.0	110	— <sup>c</sup>
	5.0	86	— <sup>c</sup>
N-ethylmaleimide	0.1	92	96
	1.0	47	86
pCMB <sup>b</sup>	0.1	37	93
	1.0	0	2
Iodoacetic acid	0.1	39	17
	1.0	27	14

<sup>a</sup> Done with S-100.<sup>b</sup> FAD, Flavin adenine dinucleotide; pCMB, *p*-chloromercuribenzoic acid.<sup>c</sup> —, Not determined; reduced glutathione (GSH) reduces sulfur nonenzymatically.

above 280 nm, the SOR is distinctly different from the siroheme sulfite reductases (11, 12). In other thiobacilli, several sulfur-oxidizing systems were found, differing in their locations, the requirement of cofactors, and their behavior under oxygen-free conditions (1, 16, 20, 22). The *Desulfurolobus* SOR did not require any cofactors and did not work without oxygen. The only enzyme system dismutating sulfur described so far was found in cell extracts of *Thiobacillus thiooxidans* producing thiosulfate and hydrogen sulfide from colloidal sulfur (21). A sulfur:ferric ion oxidoreductase purified from *Thiobacillus ferrooxidans* (18) had different properties and was not related in structure (a dimer of 2 × 23 kDa) or function to the SOR described here.

Stoichiometric calculations of the (SO<sub>3</sub><sup>2-</sup> + S<sub>2</sub>O<sub>3</sub><sup>2-</sup>)/H<sub>2</sub>S ratio were initially rendered difficult by losses of H<sub>2</sub>S as a result of nonenzymatic side reactions. Only after testing zinc ions for inhibitory effects (Table 2) did it become possible to establish the ratio of reaction products. The ratio of 1.23:1 to 1.3:1 for (SO<sub>3</sub><sup>2-</sup> + S<sub>2</sub>O<sub>3</sub><sup>2-</sup>)/H<sub>2</sub>S, found in various experiments, is similar to the 1.2:1 reported by Tano and Imai (21) for a cell-free system from *Thiobacillus thiooxidans*.

Suzuki (19) and Emmel et al. (4) proposed a highly exergonic (23) oxygenase-type reaction mechanism for sulfur-oxidizing enzymes from results of <sup>18</sup>O<sub>2</sub> incorporation experiments. The inactivity of the SOR in the absence of oxygen makes it probable that this type of reaction is also

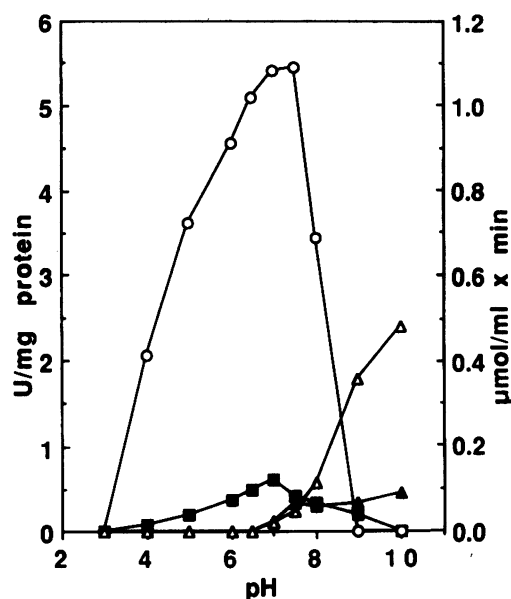


FIG. 5. pH-dependence of enzyme activity, measured at 85°C. Symbols: ○, enzymatic production of sulfite plus thiosulfate; ■, hydrogen sulfide production (left axis) or nonenzymatic controls (right axis); △, sulfite plus thiosulfate; ▲, 10× hydrogen sulfide formation.

catalyzed by the SOR, although this assumption explains neither the mechanism of hydrogen sulfide formation by the SOR nor its biological significance. Reduction of sulfur might allow the cells to utilize the high energy difference of sulfur oxidation (HSO<sub>3</sub><sup>-</sup>: ΔG°<sub>25°C</sub> = -527.81 kJ/mol [23]) for

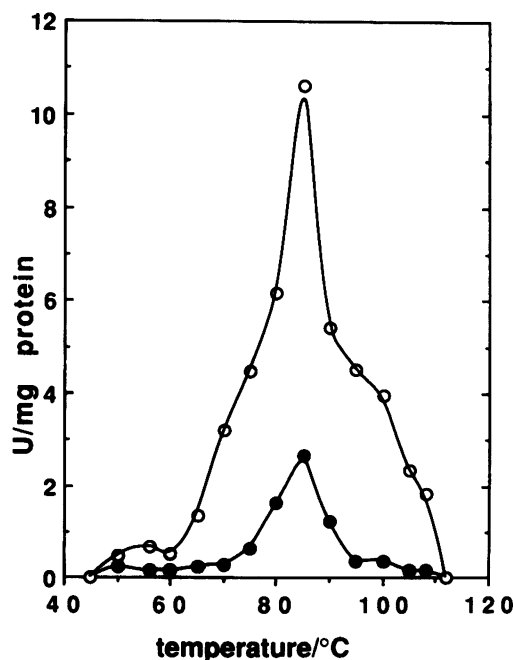


FIG. 6. Temperature dependence of activity of the purified SOR. Symbols: ○, thiosulfate plus sulfite; ●, hydrogen sulfide production. All values were calculated from the slope of the maximal increase in concentration. Formation of thiosulfate, sulfite, and hydrogen sulfide always occurred simultaneously.

ATP synthesis. From phototrophic sulfur bacteria, *c*-type cytochromes with sulfide:acceptor oxidoreductase activity coupled to the electron transport chains had been isolated (24, 25).

The SOR was not found in anaerobically growing, sulfur-reducing cells. Reaction rates of the enzyme in the absence of oxygen were close to zero (Fig. 4), showing that the reduction of sulfur with hydrogen is not catalyzed by the SOR. For this reason, it is unlikely that the SOR plays an important role in the metabolism of anaerobically growing, sulfur-reducing cells. There is presumably another membrane-bound enzyme system responsible for sulfur reduction. No such system is known for archaeobacteria, and only one is known for eubacteria (13).

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